METHOD FOR INCREASING IMMUNOGENICITY, PRODUCT OBTAINED AND PHARMACEUTICAL COMPOSITIONS

This application is a continuation of Ser. No. 08/777,293 filed Dec. 27, 1996, abandoned, and of Ser. No. 08/832,461 filed Apr. 2, 1997, abandoned, both of which are continuing applications of Ser. No. 08/488,092 filed Jun. 7, 1995, abandoned, which asserted the Aug. 31, 1994 priority date of French application Serial No. 94 10479, all of which applications are incorporated herein by reference.

The present invention relates to a method enabling the immunogenicity of an antigen to be increased, and to the use of products capable of being obtained, in particular as a vaccine.

The human cytomegalovirus (CMV), a herpesvirus in the latent state in immunocompetent individuals, is responsible for a wide variety of pathologies which are often fatal in the case of immunosuppression (foetus, graft, AIDS, leukaemia, cancer) (for review see (1)). CMV is the main source of congenital malformations of infectious origins. For all these reasons, CMV poses a serious public health problem. The battle against this virus is limited to chemotherapy with non-specific antivirals such as Ganciclovir® and Foscarnet®, the side effects and nephrotoxicity of which have been described. As regards prevention, passive immunotherapy (injection of gamma globulins) decreases the incidence of primary infection in seronegative marrow grafts. In transplant patients (marrow, kidney, liver, heart, liver allografts), CMV infection is associated with graft rejection. The recipients, under immunosuppressant (cyclosporin) are treated with Ganciclovir® during transplantation, and receive gamma globulins during the three months following surgery. The intravenous injection of immunoglobulins at high dose in these patients has permitted a reduction in the incidence of pneumonia and rejection in some cases. This gives rise to the not insignificant problem of the very high cost associated with these treatments. Opportunistic CMV infection in HIV+ individuals is among the most dramatic, and necessitates chemotherapy with antivirals.

The economies achieved by elaborating a policy of prevention of diseases associated with CMV infection in individuals at risk would be substantial. In effect, estimates of the cost associated with vaccinating an individual and with covering the attendant expenses (serological analyses, vaccine, treatment of minor side effects), carried out in the United States (2), show that this cost appears to be about 50 times lower than that of the care provided for a newborn infant who is a victim of a congenital infection. CMV infection is observed in ⅔ of renal transplant patients and at a high incidence in other transplant patients. If it is associated with complications in about ⅓ of them, the annual cost added to that of transplantation is considerable. Despite the impact on the disease of drugs such as Ganciclovir® or the injection of gamma globulins, the prevention of the primary infection and of reactivation in these patients should be a priority. The benefits of immunization are obvious from both a clinical and an economic standpoint.

The elaboration of a CMV vaccine should make it possible to curb the development of pathologies associated with congenital infections by vaccinating young women before and during pregnancy, to provide for the protection of patients awaiting transplantation and to initiate an anti-cytomegalovirus response in asymptomatic HIV+ individuals. The use of cured virus does not appear to be suitable, since viral extracts do not in general induce a cytotoxic

TCD8+ type response. Attenuated virus gives rise to the problem of the oncogenic character, latency and reactivation of the viral particles. The development of recombinant vaccines which should enable these risks to be avoided necessitates knowledge of the most immunogenic antigens or antigen fragments (epitopes) of the infectious agent. The aim of vaccination is the induction of a protective immunity. Rational approach to vaccination should involve three steps: (i) identification of the effector mechanisms responsible for protection, (ii) choice of an antigen capable of inducing a response in all individuals, and (iii) use of an administration route for the vaccine which is capable of inducing the desired type of response (humoral: antibodies, cellular: cytotoxic and helper).

The body's defence against a viral infection is brought about essentially by the development of a humoral immune response (production of neutralizing antibodies) preventing adsorption of the virus to the cell surface on the one hand, and a cellular response (cytotoxic TCD8+ cells and TCD4+ helper cells) removing infected cells and inhibiting viral replication (synthesis of cytokines (TNFα, IFN-γ, etc.)) on the other hand. As regards human cytomegalovirus, among the 200 proteins encoded by the double-stranded DNA (230 kbp), three of them are the respective major targets of these different types of response: the envelope glycoprotein gB (3), the tegument phosphoprotein pp65 (4) and the regulatory phosphoprotein IE1 (5).

While the elaboration of a CMV vaccine appears to be a necessity, there remains the general problem of the mode of conveying the recombinant antigens. The antigens introduced into synthetic structures must be capable of initiating or restoring a lasting B and T (CD4+ helper and cytotoxic CD8−) immune response, thereby effecting protection of the individuals against a primary infection, a reinfection or a reactivation of the latent virus. The activation of T cells (naive cells or memory cells) is linked to the capacity for presentation of the antigen by the antigen presenting cells, the most important of which are the dendritic cells, B lymphocytes and macrophages. In this context, the vector particles will have to permit access to the endogenous (class I, TCD8+) and exogenous (class II, TCD4+) presentation pathways.

The company Biovector Therapeutics has developed a type of structure called Biovecteur Supramoléculaire [Supramolecular Biovector] consisting of a polysaccharide core (PSC) covered with a surrounding outer layer of fatty acids (AC) or of phospholipids (SMBV), the composition of which can be varied. The polysaccharide mesh possesses an adjustable degree of crosslinking, can be functionalized (anionic or cationic radical), is very stable and permits the binding of a large amount of antigen inside the core as well as at its periphery.

Unexpectedly, the Applicant found that the combination of a protein or peptide with these vectors enabled a potentiation of the immunogenic response to be observed, relative to that brought about by administration of the antigen alone.

Accordingly, the subject of the present invention is a method for increasing the immunogenicity of an antigen, characterized in that the antigen is combined via stable interactions with a particulate vector, said vector containing:

- a non-liquid hydrophilic core
- an outer layer of compounds chosen from the group comprising phospholipids and fatty acids.

Preferably, the core consists of a matrix of naturally or chemically crosslinked polysaccharides or oligosaccharides. According to one of the aspects of the invention, ionic ligands are grafted onto the core, it being possible for this